

## AMENDMENTS

### To the Claims:

Please cancel Claims 1, 3-5, 7-8, 29 and 50 without disclaimer or prejudice, and amend Claims 9, 11, 18, 19, 28 and 51.

The currently pending and amended claims are below. Please amend the claims following, wherein the deleted matter is shown by strikethrough and the added matter is shown by underlining.

Claims 1-8 (Canceled)

9. (Currently amended) An isolated insect polynucleotide that encodes a bHLH-PAS polypeptide that is involved in binding juvenile hormone III, wherein said polynucleotide hybridizes under stringent conditions with a polynucleotide having a nucleotide sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 and SEQ ID NO:6, wherein the stringent conditions comprise hybridization in 1x SSC and 0.1% SDS at about 55°C for about 60 minutes, wherein said insect is selected from the group consisting of *Coleoptera*, *Siphonoptera*, *Orthoptera*, *Thysanoptera*, *Lepidoptera*, *Hemiptera*, and *Diptera*, and ~~An isolated polynucleotide of claim 8,~~ wherein said polynucleotide has a nucleotide sequence that encodes a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:4 and SEQ ID NO:5.
10. (Original) An isolated polynucleotide of claim 9, wherein said polynucleotide has a nucleotide sequence selected from the group consisting of SEQ ID NO:1 and SEQ ID NO:2.
11. (Currently amended) An expression vector comprising the isolated polynucleotide of claim 9 ~~claim 1~~.
12. (Original) A cultured host cell comprising the expression vector of claim 11.
13. (Original) A host cell of claim 12, wherein said host cell is selected from the group consisting of bacterial cell, yeast cell, insect cell and mammalian cell.
14. (Original) A method of producing a polypeptide, said method comprising the steps of:

- (a) culturing a host cell comprising the expression vector of claim 11, wherein said cultured host cell expresses said bHLH-Pas polypeptide, and
  - (b) isolating said polypeptide from said cultured host cell.
15. (Withdrawn) An isolated polypeptide selected from the group consisting of:
- (a) a conservative amino acid variant of SEQ ID NO:3,
  - (b) a functional fragment of a polypeptide having the amino acid sequence of SEQ ID NO:3,
  - (c) a polypeptide having an amino acid sequence of SEQ ID NO:3,
  - (d) a conservative amino acid variant of SEQ ID NO:4,
  - (e) a functional fragment of a polypeptide having the amino acid sequence of SEQ ID NO:4,
  - (f) a polypeptide having an amino acid sequence of SEQ ID NO:4, and
  - (g) a Met-JHR alternatively-spliced isoform.
16. (Withdrawn) The isolated polypeptide of claim 15, wherein said conservative amino acid variant is a polypeptide having an amino acid sequence that differs from the amino acid sequence of SEQ ID NO:3 by containing at least one amino acid substitution selected from the group consisting of (1) the substitution of an alkyl amino acid for an alkyl amino acid in SEQ ID NO:3, (2) the substitution of an aromatic amino acid for an aromatic amino acid in SEQ ID NO:3, (3) the substitution of a sulfur-containing amino acid for a sulfur-containing amino acid in SEQ ID NO:3; (4) the substitution of a hydroxy-containing amino acid for a hydroxy-containing amino acid in SEQ ID NO:3; (5) the substitution of an acidic amino acid for an acidic amino acid in SEQ ID NO:3; (6) the substitution of a basic amino acid for a basic amino acid in SEQ ID NO:3; (7) the substitution of a dibasic monocarboxylic amino acid for a dibasic monocarboxylic amino acid in SEQ ID NO:3.
17. (Withdrawn) The isolated polypeptide of claim 15, wherein said conservative amino acid variant is a polypeptide having an amino acid sequence that differs from the amino acid sequence of SEQ ID NO:4 by containing at least one amino acid substitution selected from the group consisting of (1) the substitution of an alkyl amino acid for an alkyl amino acid in SEQ ID NO:4, (2) the substitution of an aromatic amino acid for an aromatic amino acid in SEQ ID NO:4, (3) the substitution of a sulfur-containing amino acid for a sulfur-containing amino acid in SEQ ID NO:4; (4) the substitution of a hydroxy-containing amino acid for a

hydroxy-containing amino acid in SEQ ID NO:4; (5) the substitution of an acidic amino acid for an acidic amino acid in SEQ ID NO:4; (6) the substitution of a basic amino acid for a basic amino acid in SEQ ID NO:4; (7) the substitution of a dibasic monocarboxylic amino acid for a dibasic monocarboxylic amino acid in SEQ ID NO:4.

18. (Currently amended) A method for screening compounds that specifically bind with a bHLH-PAS/JHR polypeptide, comprising:

(a) incubating a test compound in a solution that comprises an isolated bHLH-PAS polypeptide, wherein said polypeptide is encoded by an isolated insect ~~the~~ polynucleotide ~~of claim 1~~, wherein said polynucleotide hybridizes under stringent conditions with a polynucleotide having a nucleotide sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 and SEQ ID NO:6, wherein said insect is selected from the group consisting of *Coleoptera*, *Siphonoptera*, *Orthoptera*, *Thysanoptera*, *Lepidoptera*, *Hemiptera*, and *Diptera*, and wherein the stringent conditions comprise hybridization in 1x SSC and 0.1% SDS at about 55C for about 60 minutes, and

(b) detecting the binding of said test compound with said polypeptide.

19. (Currently amended) A method for screening compounds that specifically bind with a complex comprising a bHLH-PAS polypeptide that is involved in binding juvenile hormone III and a heteromultimeric partner of said polypeptide, comprising:

(a) incubating a test compound in a solution that comprises an isolated bHLH-PAS polypeptide, and an isolated heteromultimeric partner of said polypeptide, wherein said polypeptide is encoded by an isolated insect ~~the~~ polynucleotide ~~of claim 1~~, wherein said polynucleotide hybridizes under stringent conditions with a polynucleotide having a nucleotide sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 and SEQ ID NO:6, wherein said insect is selected from the group consisting of *Coleoptera*, *Siphonoptera*, *Orthoptera*, *Thysanoptera*, *Lepidoptera*, *Hemiptera*, and *Diptera*, and wherein the stringent conditions comprise hybridization in 1x SSC and 0.1% SDS at about 55C for about 60 minutes, and

(b) detecting the binding of said test compound with said polypeptide.

20. (Original) The method of claim 18, wherein said test compound is detectably labeled.
21. (Original) The method of claim 20, wherein the binding of said test compound with said polypeptide is detected in step (b) using a scintillation proximity assay.
22. (Original) The method of claim 20, wherein said detectably labeled test compound comprises a detectable label selected from the group consisting of radiolabel, fluorescent label, chemiluminescent label, and bioluminescent label.
23. (Original) The method of claim 18, further comprising the step of incubating said bHLH-PAS polypeptide with a detectably labeled ligand, wherein said detectably labeled ligand is added to said solution containing said receptor at a time selected from the group consisting of
- (i) prior to step (a),
  - (ii) after step (a) and before step (b), and
  - (iii) concomitantly with the addition of said test compound.
24. (Original) The method of claim 18, wherein said detectably labeled ligand is juvenile hormone or a juvenile hormone analog, and wherein said detectable label is selected from the group consisting of radiolabel, fluorescent label, chemiluminescent label, and bioluminescent label.
25. (Original) The method of claim 24, wherein said detectably labeled juvenile hormone is [<sup>3</sup>H] 10R-juvenile hormone III.
26. (Original) The method of claim 24, wherein said detectably labeled juvenile hormone is [<sup>3</sup>H] methoprene.
27. (Original) The method of claim 18, further comprising the step of incubating said bHLH-PAS polypeptide with a detectably labeled photoaffinity analog of juvenile hormone after step (a) and before step (b).
28. (Currently amended) The method of claim 18, wherein said bHLH-PAS polypeptide is selected from the group consisting of:
- (a) a conservative amino acid variant of a polypeptide encoded by a polynucleotide as defined in SEQ ID NO:3,
  - (b) a functional fragment of a polypeptide encoded by a polynucleotide as defined in ~~having the amino acid sequence of~~ SEQ ID NO:3,

- (c) a polypeptide encoded by a polynucleotide as defined in ~~having an amino acid sequence of SEQ ID NO:3,~~
- (d) a conservative amino acid variant of SEQ ID NO:4,
- (e) a functional fragment of a polypeptide having the amino acid sequence of SEQ ID NO:4,  
and
- (f) a polypeptide having an amino acid sequence of SEQ ID NO:4, ~~and~~
- ~~(g) a Met-JHR alternatively spliced isoform.~~

29. (Canceled)

30. (Withdrawn) A method for detecting JH-resistant individuals in an insect population, said method comprising:

- (a) obtaining a representative biological sample of said population; and
- (b) detecting a nucleic acid sequence in said sample that corresponds to a predetermined sequence within a polynucleotide encoding a bHLH-PAS polypeptide that is altered in JH analog-resistant individuals, wherein said polypeptide is involved in binding juvenile hormone III.

31. (Withdrawn) A method according to claim 30, wherein said detecting step comprises:

- (i) amplifying a nucleic acid sequence from said sample, wherein said sequence corresponds to a predetermined sequence within a polynucleotide encoding a bHLH-PAS/JHR polypeptide and wherein said sequence comprises at least one RFLP characteristic of JH analog resistance;
- (ii) incubating said amplified nucleic acid with at least one predetermined restriction endonuclease, to form fragments;
- (iii) size-separating said fragments to form a detectable pattern; and
- (iv) comparing said pattern with a predetermined pattern obtained from JH analog-resistant individuals to detect the appearance of one or more RFLP characteristic of JH analog resistance.

32. (Withdrawn) An in vivo method for screening compounds that specifically bind with a bHLH-PAS polypeptide that is involved in binding juvenile hormone III, comprising:

- (a) providing a host cell comprising (1) DNA encoding a fusion polypeptide comprising said bHLH-PAS polypeptide and a second polypeptide comprising a DNA binding domain, and (2) a reporter gene under the control of a minimal promoter driven by the response element for said second polypeptide;
  - (b) incubating a test compound with said host cell; and
  - (c) detecting the binding of the test compound to said bHLH-PAS polypeptide by monitoring expression of the reporter gene.
33. (Withdrawn) An in vivo method for screening compounds that specifically bind with a bHLH-PAS polypeptide that is involved in binding juvenile hormone III, comprising the steps of:
- (a) providing a host cell comprising (1) DNA encoding a fusion polypeptide comprising said bHLH-PAS polypeptide and a second polypeptide comprising a DNA binding domain; (2) a reporter gene under the control of a minimal promoter driven by the response element for said second polypeptide; and (3) DNA encoding a polypeptide that is a heterodimeric partner of said bHLH-PAS polypeptide;
  - (b) incubating a test compound with said host cell; and
  - (c) detecting the binding of the test compound to said bHLH-PAS polypeptide by monitoring expression of the reporter gene.
34. (Withdrawn) An in vivo method for screening compounds that specifically bind to a multimeric complex comprising a bHLH-PAS polypeptide that is involved in binding juvenile hormone III and the heteromultimeric partner of said polypeptide, comprising the steps of:
- (a) providing a host cell comprising (1) DNA encoding a fusion polypeptide comprising said bHLH-PAS polypeptide and a second polypeptide comprising a DNA binding domain; (2) DNA encoding a heteromultimeric partner of said bHLH-PAS polypeptide and the activation domain of said second polypeptide, and (3) a reporter gene under the control of a minimal promoter driven by the response element for said second polypeptide;
  - (b) incubating a test compound with said host cell; and



(c) detecting the binding of the test compound to said complex by monitoring expression of the reporter gene.

35. (Withdrawn) An in vivo method for screening compounds that specifically bind to a multimeric complex comprising a bHLH-PAS polypeptide that is involved in binding juvenile hormone III and the heteromultimeric partner of said polypeptide, comprising the steps of:

(a) providing a host cell comprising (1) DNA encoding a fusion polypeptide comprising bHLH-PAS polypeptide and the activation domain of a second polypeptide; (2) DNA encoding a heteromultimeric partner of said bHLH-PAS polypeptide and the DNA binding domain of said second polypeptide, and (3) a reporter gene under the control of a minimal promoter driven by the response element for said second polypeptide;

(b) incubating a test compound with said host cell; and

(c) detecting the binding of the test compound to said complex by monitoring expression of the reporter gene.

36. (Withdrawn) An in vivo method for screening compounds that specifically bind with a bHLH-PAS polypeptide that is involved in binding juvenile hormone III, comprising the steps of:

(a) providing a host cell comprising (1) DNA encoding a fusion polypeptide comprising bHLH-PAS polypeptide and the DNA binding domain of a second polypeptide; (2) DNA encoding a bHLH-PAS polypeptide and the activation domain of said second polypeptide, and (3) a reporter gene under the control of a minimal promoter driven by the response element for said second polypeptide;

(b) incubating a test compound with said host cell; and

(c) detecting the binding of the test compound with said bHLH-PAS polypeptide by monitoring expression of the reporter gene.

37. (Withdrawn) A method according to any of claims 32, wherein said host cell is selected from the group of an insect cell, a yeast cell, and a mammalian cell.

38. (Withdrawn) A method according to any of claims 33, wherein said host cell is selected from the group of an insect cell, a yeast cell, and a mammalian cell.

39. (Withdrawn) A method according to any of claims 34, wherein said host cell is selected from the group of an insect cell, a yeast cell, and a mammalian cell.

40. (Withdrawn) A method according to any of claims 35, wherein said host cell is selected from the group of an insect cell, a yeast cell, and a mammalian cell.

41. (Withdrawn) A method according to any of claims 36, wherein said host cell is selected from the group of an insect cell, a yeast cell, and a mammalian cell.

Claims 42-43 (Canceled)

44. (Previously presented) An isolated polynucleotide which comprises the sequence of nucleotide 1 through nucleotide 1291 of SEQ ID NO:1.

45. (Previously presented) An isolated polynucleotide which comprises the sequence of nucleotide 1 through nucleotide 1513 of SEQ ID NO:1.

46. (Previously presented) An isolated polynucleotide which comprises the sequence of nucleotide 3733 through nucleotide 6235 of SEQ ID NO:1.

47. (Previously presented) An isolated polynucleotide which comprises the sequence of nucleotide 4302 through nucleotide 6235 of SEQ ID NO:1.

Claims 48-50 (Canceled)

51. (Currently amended) An isolated insect polynucleotide that encodes a bHLH-PAS polypeptide that is involved in binding juvenile hormone III ~~polynucleotide as claimed in claim 1,~~ wherein said polynucleotide hybridizes under stringent conditions with a polynucleotide having a nucleotide sequence of SEQ ID NO:7, wherein the stringent conditions comprise hybridization in 1xSSC and 0.1% SDS at about 55°C for about 60 minutes, and wherein said insect is selected from the group consisting of *Coleoptera*, *Siphonoptera*, *Orthoptera*, *Thysanoptera*, *Lepidoptera*, *Hemiptera*, and *Diptera*.